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The mode of action of anticholinergics in asthma

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Pre-clinical data suggest that anticholinergics can reduce acetylcholine-induced airway inflammation and remodelling <http://ow.ly/xqAQ30loP8F>

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ABSTRACT Acetylcholine binds to muscarinic receptors to play a key role in the pathophysiology of asthma, leading to bronchoconstriction, increased mucus secretion, inflammation and airway remodelling. Anticholinergics are muscarinic receptor antagonists that are used in the treatment of chronic obstructive pulmonary disease and asthma. Recent *in vivo* and *in vitro* data have increased our understanding of how acetylcholine contributes to the disease manifestations of asthma, as well as elucidating the mechanism of action of anticholinergics. This review assesses the latest literature on acetylcholine in asthma pathophysiology, with a closer look at its role in airway inflammation and remodelling. New insights into the mechanism of action of anticholinergics, their effects on airway remodelling, and a review of the efficacy and safety of long-acting anticholinergics in asthma treatment will also be covered, including a summary of the latest clinical trial data.

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Introduction

Acetylcholine is the predominant parasympathetic neurotransmitter in the airways [1], and plays a key role in the pathophysiology of obstructive airway diseases, such as asthma, through bronchial smooth muscle contraction and mucus secretion [2]. Pre-clinical evidence supports an additional role in airway inflammation and remodelling [3]. Acetylcholine binds to muscarinic receptors [2, 3], making these receptors an attractive target for respiratory disease therapy, such as in asthma.

Anticholinergics are muscarinic receptor antagonists that have been used to treat chronic obstructive pulmonary disease (COPD) for several years and are now used as add-on treatment in asthma. In this review, we assess the latest literature on acetylcholine in asthma pathophysiology, including its role in airway inflammation and remodelling. We also review new insights into the mechanism of action of anticholinergics and their effects on airway remodelling. A comparison of the efficacy and safety of long-acting anticholinergics in asthma treatment will also be covered, with a summary of the latest clinical trial data.

The role of acetylcholine in asthma pathophysiology

Increased acetylcholine signalling in asthma

Research has shown that parasympathetic neuronal activity, through acetylcholine signalling, is increased in the pathophysiology of asthma [2, 3]. Acetylcholine is released from airway neurons and non-neuronal cells such as airway epithelial cells [4]. Other non-neuronal sources include inflammatory cells [2]. Acetylcholine binds to airway muscarinic receptors to trigger smooth muscle contraction and mucus secretion (figure 1) [2, 3]. There are five identified muscarinic receptors that belong to the G-protein-coupled receptor family [5]; however, only M_1 , M_2 and M_3 receptors have been shown to play major roles in airway physiology, and in diseases such as asthma and COPD [5]. M_1 receptors are expressed by epithelial cells and in the ganglia; they regulate electrolyte and water secretion, and aid parasympathetic neurotransmission, respectively [6]. M_2 receptors are expressed in airway smooth muscle and on parasympathetic neurons; they have a very limited role in contraction on airway smooth muscle. However, M_2 receptors act as autoreceptors on parasympathetic neurons to limit acetylcholine release, thus limiting vagal reflex-induced bronchoconstriction and mucus secretion [2, 7]. M_3 receptors are the primary receptor subtype for bronchial smooth muscle contraction, and are found in airway smooth muscle and submucosal glands [2, 5, 8].

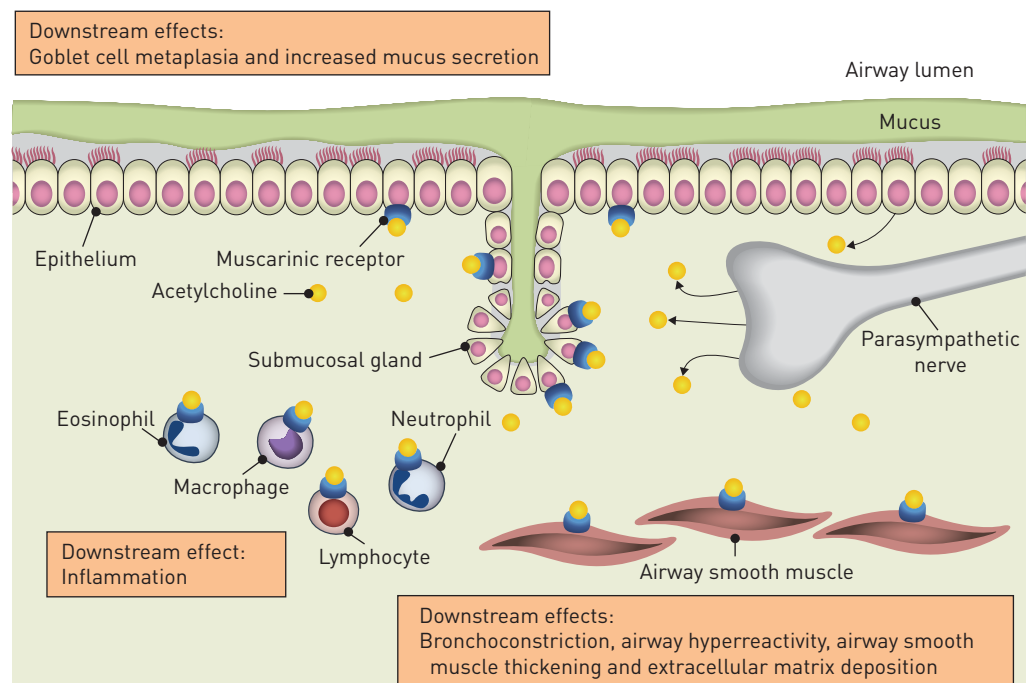


FIGURE 1 A summary of the role of acetylcholine in asthma pathophysiology. Acetylcholine is the predominant parasympathetic neurotransmitter in the airways. It is released from airway neurons and non-neuronal cells, such as airway epithelial cells, and binds to muscarinic M_1 , M_2 and M_3 receptors. These receptors are found on airway epithelial cells, smooth muscle cells and submucosal glands. Binding of acetylcholine to the muscarinic receptors triggers a host of downstream effects associated with the pathophysiology of asthma.

Several mechanisms account for increased neural activity in asthmatic airways [2]. Established mechanisms include loss of epithelial barrier function due to an inflammatory local tissue microenvironment, which exposes the neurons to the airway lumen [2]. Inflammatory mediators and even direct contact of airway nerves with eosinophils can then activate the exposed neurons to trigger vagal reflex-mediated bronchoconstriction [9]. This bronchoconstriction is compounded by the dysfunction of M_2 autoreceptors; this results in increased acetylcholine release, leading to airway hyperreactivity [5]. M_2 receptor dysfunction has been shown in animal model studies of airway disease following exposure to allergens, ozone and viral infections [10]. In support of a role in asthma, the M_2 agonist pilocarpine protects from reflex bronchoconstriction in normal subjects, but not in those with asthma [11]. M_2 receptor dysfunction is thought to be driven by eosinophils and the secretion of major basic protein [10, 12]. In addition, tumour necrosis factor- α appears to play a key role in driving M_2 autoreceptor dysfunction in animal models of ozone- and virus-induced airway hyperreactivity [13, 14]. The increase in acetylcholine signalling on M_1 and M_3 receptors, and the M_2 receptor dysfunction, may all contribute to the increased bronchoconstriction, mucus secretion, inflammation and airway remodelling, as discussed in the following sections.

An exciting, novel development in this area of research is neuronal plasticity and remodelling, which may underpin persistent changes in cholinergic signalling in asthma. Airway neurons have received little attention in studies into mechanisms of tissue remodelling in asthma, yet seem to switch to a cholinergic isotype and branch more excessively in response to inflammatory insults, including allergens and eosinophilic inflammation [15, 16]. Intriguingly, a recent study showed that such neuronal plasticity may be a feature of early-life exposure to allergens, following which the neurotrophin NT-4 mediates neuronal remodelling and persistent airway hyperresponsiveness beyond the immediate period of allergen exposure [17]. In light of the observation that single nucleotide polymorphisms (SNPs) in genes that encode neurotrophic factors, such as brain-derived neurotrophic factor, may be associated with asthma and allergic rhinitis [18], pre-clinical studies that investigate the molecular control of this response and studies that characterise the pathological features of neuronal remodelling in patients with asthma are clearly needed.

Downstream effects of increased acetylcholine signalling: mechanisms and therapeutic implications

Bronchoconstriction

The increased vagal activity brought on by increased acetylcholine signalling contributes to bronchoconstriction; in fact, the improvement in forced expiratory volume in 1 s (FEV₁) in response to tiotropium is fairly similar to that induced by the β_2 -agonist salmeterol in mild-to-moderate asthma patients [19]. This is intriguing, as the long-acting anticholinergic blocks a single mediator only, whereas the β_2 -agonist is a functional antagonist of contraction, irrespective of the mediator that caused the effect. Observations from pre-clinical studies in animals show that this may be explained by the use of the cholinergic system by inflammatory mediators and bronchoconstrictors even if these do not directly act on muscarinic receptors. For example, results from an *in vivo* study of allergen-induced bronchial hyperreactivity in sensitised guinea pigs show that vagally derived acetylcholine contributes to histamine-induced bronchoconstriction in allergen-challenged animals on a selective basis [20]. Thromboxane A_2 , a potent mediator of airway constriction, is dependent on parasympathetic signalling in both healthy and inflamed airways [21]. Binding of thromboxane A_2 to its receptors is thought to substantially increase the release of acetylcholine [21]. Increased vagal activity is also thought to contribute to the early asthmatic reaction and late asthmatic reaction (LAR). Data from pre-clinical *in vivo* models suggest allergens activate airway sensory nerves, at least in part *via* transient receptor potential ankyrin-1 channels [22]. This initiates a central reflex event leading to acetylcholine-induced bronchoconstriction, which may be responsible for the LAR [22]. As such, the cholinergic reflex arc promotes bronchoconstriction to histamine, inflammatory mediators and allergens.

In a guinea pig model of acute allergic asthma, tiotropium even reverses and protects against allergen-induced airway hyperresponsiveness [23]. A clinical study comparing the effectiveness of indacaterol/tiotropium and indacaterol (both in combination with inhaled corticosteroids (ICSs)) on mannitol-induced airway responsiveness found no additional effect of tiotropium on top of indacaterol on mannitol median effective dose (ED₅₀) [24], whereas sulfur dioxide-induced airway hyperresponsiveness in asthmatic subjects is subject to cholinergic control [11]. Thus, whereas it is clear that acetylcholine contributes to bronchoconstriction in asthma, the contribution of the cholinergic reflex arc to (the development of) airway hyperresponsiveness in asthma is not extensively reported and needs further study.

Increased mucus secretion

Acetylcholine-induced mucus secretion is also a key feature of asthma. Mucus glands are innervated by parasympathetic nerves and release mucus in response to electrical field stimulation [25]. Goblet cells do

express muscarinic receptors, but require relatively high concentrations of muscarinic agonist to promote secretory activity [26]. An interesting novel finding is that, independent of any effects on airway inflammation, muscarinic receptors may also control goblet cell differentiation. Repeated methacholine challenges promote the presence of mucus-positive cells in the airway epithelium of patients with mild asthma [27]. In a study of human airway epithelial cells cultured on an air-liquid interface, tiotropium was shown to attenuate goblet cell metaplasia induced by interleukin (IL)-13 [28]. In addition, tiotropium reversed established goblet cell hyperplasia [28]; interestingly, no exogenous muscarinic receptor agonist was added to the system, indicating non-neuronal acetylcholine produced by the epithelial cells themselves contributes to goblet cell differentiation. Mechanistically, this was dependent on the regulation of the FoxA2 and FoxA3 transcription factors that regulate mucus cell differentiation by IL-13, which was prevented by tiotropium. KOMIYA *et al.* [29] found that the anticholinergic tiotropium had no effect on goblet cell metaplasia or mucin secretion induced by IL-13, but decreased mucin secretion stimulated by neutrophil elastase. IL-17-induced acetylcholine production promoted mucus secretion for the bronchial epithelial cell line 16-HBE [30].

In vivo data have shown that when sensitised M₃ receptor-deficient mice were exposed to allergen challenge, they had a 30% lower increase in goblet cells compared with wild-type mice ($p < 0.05$) [31]. They also showed a significantly lower increase in the mucus-producing gene *MUC5AC* compared with wild-type mice (35%; $p < 0.05$) [31]. Treatment with tiotropium in sensitised guinea pigs also completely prevented allergen-induced mucus gland hypertrophy [32], a finding that was also reported for house dust mite-induced responses in mice [33]. Repeated exposure of mice to cholinergic agonists also promoted goblet cell presence in the airway epithelium [34].

Thus, whereas it appears that goblet cell differentiation of airway epithelium is indeed subject to cholinergic control, the underlying mechanisms are not yet fully established. Cholinergic receptors are G_q-coupled receptors and therefore not presumed to directly couple to STAT (signal transducer and activator of transcription) pathway activation, so the impact on IL-13, IL-17 and neutrophil elastase signalling is unlikely to be through direct modulation of that activity [2]. An additional area that remains unexplored is whether goblet cell hyperplasia in asthmatic patients is sensitive to anticholinergic treatment.

Airway inflammation

In addition to bronchoconstriction and mucus secretion, acetylcholine also contributes to airway inflammation, although at present this has only been reported in pre-clinical models and is yet to be confirmed in asthmatic subjects. *In vitro*, acetylcholine signalling leads to the release of eosinophil chemotactic activity from bovine bronchial epithelial cells (BECs) in a dose- and time-dependent manner [35]. Of interest, eosinophils have been shown to gather around the nerves in airways of sensitised guinea pigs and humans who have died of fatal asthma [36]. Other data suggest that acetylcholine signalling polarises dendritic cells towards a T-helper cell type 2 (Th2) profile [37]. Incubation of dendritic cells with acetylcholine stimulated production of two chemokines that recruit Th2 cells to allergic inflammation sites (macrophage-derived chemokine, and thymus and activation-regulated chemokine) [37]. Mechanistically, the effect is not fully clear at this stage, but regulation of the pro-inflammatory transcription factor NF- κ B and of protein kinase C (PKC) by muscarinic receptors may play a role [38].

In vivo, anticholinergics can reduce the acetylcholine-induced inflammatory response by inhibiting the release of chemokines and recruitment of inflammatory cells [39]. Aclidinium, a long-acting anticholinergic, has been shown to reduce both allergen-induced and methacholine-induced airway hyperresponsiveness in both naive and sensitised mice [40]. There was also a substantial decrease ($56 \pm 4\%$) in allergen-induced eosinophilia with aclidinium treatment, suggesting an anti-inflammatory role [40]. Similarly, tiotropium has shown anti-inflammatory properties: in a rat model of resistive breathing, tiotropium was shown to attenuate the increase in bronchoalveolar lavage neutrophil number, IL-1 β and IL-6 levels, and lung injury score [41]. Tiotropium was also shown to reduce inflammation in a dose-dependent manner in sensitised mice [33]. Another study of sensitised mice showed a significant reduction in airway inflammation with tiotropium [42]. Furthermore, tiotropium reduces eosinophilic inflammation in chronically challenged guinea pigs to a similar extent as budesonide [32] and tiotropium synergises with ciclesonide in reducing allergen-induced inflammation in the same model [43].

An intriguing, novel finding is that cholinergic nerves may release the recently identified neuromedin U, which participates in Th2-type inflammation by directly activating eosinophils and potentially type 2 innate lymphoid cells [44–46]. In light of the aforementioned regulation of neuronal plasticity in asthma, this is an exciting new development linking cholinergic regulation to airway inflammation that needs to be followed up to establish its importance in asthma. Immunomodulatory effects of anticholinergics could prevent asthma exacerbations by reducing inflammation and mucus production in the airways, and indeed

tiotropium was reported to reduce exacerbations clinically [33]. Whether this is truly due to anti-inflammatory activity by anticholinergics is a major open question that remains unanswered.

Airway remodelling

Airway remodelling involves structural changes to the airways, such as goblet cell metaplasia, airway smooth muscle thickening and extracellular matrix deposition [28, 47]. Several pathways contribute to remodelling, including growth factors, mediators and extracellular matrix proteins present in the airway wall [48]. In addition, there is some evidence indicating cholinergic control of airway remodelling in asthma patients. For example, tiotropium reduces airway wall dimensions in combination with long-acting β_2 -agonist (LABA) and ICS therapy in patients with asthma, as assessed by quantitative computed tomography [49]. Furthermore, repeated bronchoconstriction with either dust mite or methacholine challenge in patients with asthma increased the percentage of epithelium staining for mucus-producing cells and subepithelial markers for airway remodelling. The fact that this change was not seen in the control group, and was reversible with albuterol treatment, suggests that bronchoconstriction can trigger excess mucus production, leading to further airway obstruction [27]. Interestingly, eosinophilic inflammation was only seen in patients who received the dust mite allergen. This supports the idea that acetylcholine-induced bronchoconstriction alone can induce airway remodelling [27].

In vitro and animal model studies indicate that these changes are mediated mostly by M_3 receptors, which in turn are activated by acetylcholine. *In vitro* data have shown that downstream signalling from muscarinic receptors triggers glycogen synthase kinase (GSK)-3 inhibition, which, in its active state, acts to repress airway smooth muscle proliferation. This suggests a possible mechanism for the accumulation of smooth muscle in airway remodelling [50]. Muscarinic receptors control contractile protein accumulation in combination with transforming growth factor (TGF)- β as well *via* such a GSK-3-dependent mechanism [51], whereas the cooperative regulation of extracellular matrix protein production by muscarinic receptors and TGF- β appears to involve M_2 receptors [52]. An *in vitro* model of guinea pig lung slices found that methacholine-induced bronchoconstriction leads to contractile protein expression, such as smooth muscle myosin. This was mediated by the release of bioactive TGF- β [53], thought to be responsible for several features of airway remodelling, such as myofibroblast transformation, enhanced collagen synthesis and deposition in the sub-basement membrane, and increased expression of smooth muscle contractile protein [47, 54]. The release of bioactive TGF- β in response to methacholine [55] supports these findings. Further evidence suggests that it is the mechanical effects of acetylcholine-mediated bronchoconstriction that causes airway remodelling [3, 56, 57]. BECs obtained from volunteers with asthma showed increased secretion of TGF- β and granulocyte-macrophage colony-stimulating factor when subjected to compressive forces when compared with BECs from volunteers without asthma [47].

In vivo data showed that wild-type mice had a 1.7-fold increase in staining for α -smooth muscle actin following allergen challenge; this increase was completely absent in mice deficient in M_3 receptors [31]. This study did not find any stimulatory role for M_3 receptors in allergic inflammation, thus suggesting that acetylcholine-induced remodelling can be independent of inflammation [31].

Use of tiotropium in sensitised mice resulted in reductions in goblet cell metaplasia, airway smooth muscle thickness and levels of TGF- β , suggesting a role for tiotropium in reduction of airway remodelling and hyperresponsiveness [42]. This is further supported by a study of tiotropium in sensitised guinea pigs, which resulted in $\leq 75\%$ inhibition in airway smooth muscle mass [32]. Combination therapy of tiotropium with ciclesonide in a guinea pig model of chronic asthma also significantly reduced allergen-induced airway smooth muscle mass by 81% ($p < 0.05$) [43].

These data add insight into the role of bronchoconstriction in airway remodelling. However, a recent study indicates that repeated exposure of mice to methacholine induces changes in goblet cell hyperplasia and macrophage presence, but does not impact airway responsiveness [34]. Clearly, further studies are needed to investigate in more detail the hypothesis that bronchoconstriction can drive airway remodelling independently from inflammation. In particular, the underlying mechanisms need further clarification to explain the relatively diverse functional and pathological outcomes in the aforementioned different experimental models.

Anticholinergics in asthma

There is extensive experience of anticholinergic use in obstructive respiratory diseases, as they have been approved for use in COPD for many years [58]. There are five anticholinergics currently licensed for use in COPD: ipratropium [59], aclidinium [60], glycopyrronium (also known as glycopyrrolate) [61], umeclidinium [62] and tiotropium [63]. However, only two anticholinergics have been approved for use in asthma: ipratropium and tiotropium. Ipratropium is a short-acting anticholinergic approved for use in the treatment of reversible airways obstruction in acute and chronic asthma in combination with β_2 -agonists

[5, 59], whereas tiotropium is the only long-acting anticholinergic approved for use in asthma as add-on therapy to ICS and a LABA [63].

Properties of anticholinergics

Anticholinergics are reversible competitive inhibitors of M_1 , M_2 and M_3 receptors [6], and have been shown to have similar binding affinity for all five muscarinic receptor subtypes [64]. The time spent at the muscarinic receptors determines the duration of action of each drug. For example, the long-acting anticholinergics show kinetic selectivity for M_3 receptors over M_2 receptors (table 1), as they dissociate more slowly from M_3 receptors than M_2 receptors [6, 65, 66]. *In vitro* data have shown that aclidinium dissociates slightly faster from M_2 and M_3 receptors than tiotropium, but more slowly than ipratropium and glycopyrronium (residence half-lives at M_3 receptors are shown in table 1) [65]. In a separate set of experiments conducted by SALMON *et al.* [67], binding studies conducted using Chinese hamster ovary cells expressing human M_1 – M_5 receptor subtypes showed that the pK_i for umeclidinium was 9.8 (M_1), 9.8 (M_2), 10.2 (M_3), 10.3 (M_4) and 9.9 (M_5). Dissociation of umeclidinium from the M_3 receptor was slower than that for the M_2 receptor. The half-life of tiotropium in this study was longer than that of umeclidinium for both the M_2 receptor (39.2 *versus* 9.4 min, tiotropium and umeclidinium, respectively) and M_3 receptor (272.8 *versus* 82.2 min, tiotropium and umeclidinium, respectively) [67]. CASAROSA *et al.* [65] also reported that tiotropium dissociates more slowly from the M_3 than the M_2 receptor; however, the half-lives were 27 and 2.6 h, respectively. The differences in half-lives observed in these two studies may have been due to methodological differences employed in the two studies.

Clinical data of anticholinergics in airway inflammation and remodelling

There are limited clinical data to explain the role of anticholinergics in airway inflammation and remodelling in patients with asthma. A clinical study in patients with symptomatic asthma receiving ICS and LABA assessed the effect of tiotropium on airway geometry and inflammation. Tiotropium significantly decreased airway wall area and thickness, corrected for body surface area ($p < 0.05$ for both), and improved airflow obstruction. These data suggest a potential protective effect of tiotropium against bronchoconstriction and airway remodelling [49]. Patients with severe asthma have shown improved symptoms and lung function with tiotropium add-on to ICS, which suggests a role in reducing airway inflammation [68]. The clinical data of anticholinergics in asthma are summarised later in this review.

Comparison of mechanism of action: anticholinergics, short-acting β_2 -agonists and long-acting β_2 -agonists

Anticholinergics have a different mechanism of action compared with short-acting β_2 -agonists (SABAs) and LABAs, which bind to airway β_2 -receptors to trigger smooth muscle relaxation [69, 70]. However, data suggest concomitant use of anticholinergics with β_2 -agonists can enhance the β_2 -agonist-induced bronchodilation *via* intracellular processes [71]. Glycopyrronium was shown to enhance muscarinic contraction with SABAs by decreasing Ca^{2+} sensitisation and dynamics through PKC and calcium-activated potassium (K_{Ca}) channels [71]. This suggests that PKC and K_{Ca} channels may be involved in the cross-talk between anticholinergics and β_2 -agonists. Studies assessing aclidinium and formoterol fumarate, and glycopyrronium and indacaterol fumarate, have shown enhanced benefits on airway smooth muscle relaxation in human isolated bronchi [72, 73]. LABA and anticholinergic combination therapy may also mitigate daily variations in sympathetic and parasympathetic activity. A clinical study in patients with COPD showed that tiotropium was associated with sustained improvements in lung function throughout 24 h, without affecting circadian variability [74]. These data show that dual

TABLE 1 Binding affinities (pK_i) and dissociation half-lives ($t_{1/2}$) of anticholinergics against muscarinic M_1 , M_2 and M_3 receptor subtypes

	pK_i			$t_{1/2}$ h		
	M_1	M_2	M_3	M_1	M_2	M_3
Ipratropium	9.40	9.53	9.58	0.1	0.03	0.22
Aclidinium	10.78	10.68	10.74	6.4	1.8	10.7
Glycopyrronium	10.09	9.67	10.04	2.0	0.37	6.1
Tiotropium	10.80	10.69	11.02	10.5	2.6	27

Dissociation constants determined by analysing competition kinetics curves in the presence of [N -methyl- 3H]scopolamine and different concentrations of unlabelled antagonist. Data from [65].

bronchodilation with anticholinergic add-on therapy and β_2 -agonism has a greater benefit than single bronchodilation. Furthermore, combination therapy of ipratropium on top of salbutamol prolongs the duration of action of the bronchodilator effect [75]. These and other considerations, such as the frequent use of ipratropium (4 puffs per day), have triggered studies into the role of long-acting anticholinergics in COPD and, more recently, in asthma.

Use of short-acting anticholinergics in asthma

Ipratropium is a non-selective antagonist of muscarinic receptors [76], approved for use in acute and chronic asthma in combination with β_2 -agonists [5, 59]. It can be used as an alternative reliever agent for patients with asthma who are refractory to β_2 -agonists [77]. However, data suggest that it is not as effective as SABAs; a study of 188 patients with chronic bronchitis ($n=113$) or asthma ($n=75$) found that asthma patients were more likely to respond better to salbutamol than to ipratropium [78].

Use of long-acting anticholinergics in asthma

Acclidinium

Acclidinium is licensed for use in COPD only [60]. At the time of writing, there were no registered clinical trials for acclidinium in asthma and so this anticholinergic will not be discussed further in this review.

Glycopyrronium

Glycopyrronium is also licensed for use in COPD only [61], but there have been studies assessing its use in asthma. In patients with mild-to-moderate asthma, glycopyrronium provided significantly more protection against methacholine-induced bronchoconstriction than placebo ($p<0.002$) [79]. Glycopyrronium also provided bronchodilation for up to 30 h after each inhalation [79]. There are currently two clinical trials assessing glycopyrronium use in patients with asthma: one study is assessing the bronchodilator effects and safety of two doses of glycopyrronium (25 μg and 50 μg) in adults with asthma receiving ICS/LABA (ClinicalTrials.gov identifier NCT03137784; completion date: December 2017); the other study is assessing triple therapy of glycopyrronium, indacaterol and mometasone furoate in patients with uncontrolled asthma despite ICS/LABA treatment (ClinicalTrials.gov identifier NCT03158311; estimated completion date: June 2019).

Umeclidinium

Umeclidinium is licensed for use in COPD, but is not approved for use in asthma [62, 80]. A phase II study found a modest improvement in trough FEV₁ with umeclidinium monotherapy in patients with asthma not receiving ICS [81]. However, these improvements were not dose-related or consistent in magnitude, meaning that these data do not conclusively show a therapeutic benefit with umeclidinium monotherapy. Another phase II study evaluated the dose response, efficacy and safety of several doses of umeclidinium in combination with fluticasone furoate in patients with symptomatic asthma despite ICS therapy [82]. There was a significant improvement in trough FEV₁ with the combination therapy (highest doses of umeclidinium bromide) compared with fluticasone furoate alone ($p=0.018$) [82]. There are currently two ongoing clinical trials assessing fixed-dose combination of umeclidinium, fluticasone furoate and vilanterol in patients with asthma (ClinicalTrials.gov identifiers NCT03184987 and NCT02924688; estimated completion dates: June 2019 and February 2019, respectively).

Tiotropium

Tiotropium is licensed for use in COPD as maintenance therapy, and in asthma as add-on therapy to ICS/LABA in adults, adolescents and children aged ≥ 6 years [63, 83]. In February 2017, the US Food and Drug Administration approved tiotropium Respimat for use in children with asthma aged ≥ 6 years [83]. There is an extensive clinical trial programme assessing the use of tiotropium in adults, adolescents and children with asthma. Tiotropium 5 μg added on to at least ICS and LABA therapy in adult patients with poorly controlled symptomatic asthma resulted in an improvement of up to 154 mL in peak FEV₁ ($p<0.001$), with a 21% risk reduction for severe asthma exacerbation ($p=0.03$) [84]. A subgroup analysis also reported a reduced risk of severe asthma exacerbations, asthma worsening and improved asthma control responder rate regardless of baseline clinical features (sex, age, body mass index, disease duration, age of onset and smoking status) [85]. A pooled safety analysis of seven randomised, double-blind, placebo-controlled studies (both phase II and III) found that both 2.5 and 5 μg doses of tiotropium had comparable safety and tolerability with placebo; the frequencies of patients reporting any type of adverse effect were 57.1% versus 55.1% and 60.8% versus 62.5%, respectively [86]. Several studies in adolescents and children have also shown significant improvements in lung function, with a comparable safety profile to placebo [87–91]. Overall, these data show that tiotropium is efficacious and has a favourable safety profile across a range of asthma severities in adults, adolescents and children [19, 84–89, 92, 93].

Who can benefit from long-acting anticholinergics?

There are limited step-up treatment options for patients who continue to have frequent symptoms and exacerbations while taking combination ICS/LABA treatment [94]. In addition, there are safety concerns for regular use of β_2 -agonists in some patients, particularly those with the SNP in the β_2 -adrenergic receptor gene *ADRB2* genotype [76, 95]. Some patients may associate ICSs with systemic side-effects, particularly in children, such as reduced bone density and growth [96]. Long-acting anticholinergics can be a suitable add-on therapy for patients who remain symptomatic despite ICS and LABA therapy or who are unable to receive conventional therapies. The benefits seen with tiotropium add-on therapy in the subgroup analysis in patients with poorly controlled symptomatic asthma also suggest that a broad range of patients can benefit from anticholinergics, irrespective of baseline characteristics [85].

Conclusions

Acetylcholine plays an important role in the pathophysiology of asthma *via* binding to airway muscarinic receptors to trigger bronchoconstriction, mucus secretion and inflammation, while pre-clinical data have highlighted the importance of cholinergic-mediated bronchoconstriction in airway remodelling. Anticholinergics antagonise the parasympathetic effects of acetylcholine, thus providing therapeutic benefit *via* a supplementary mechanism to ICS and LABA effects in asthma. Clinical data have shown that long-acting anticholinergics are well tolerated, with infrequent and mild side-effects. The extensive clinical trial data of tiotropium, particularly in asthma studies, demonstrate clinical efficacy and treatment benefit as an add-on therapy in symptomatic asthma across a range of age groups and asthma severities.

Future studies are needed, however, to clarify the cholinergic control of asthma pathophysiology in more detail. In particular, areas that require further investigation are neuronal plasticity in asthma and its contribution to airway hyperresponsiveness and remodelling; the anti-inflammatory effects of anticholinergics in asthma patients; and the mechanisms that underpin the cholinergic control of airway inflammation and remodelling, in particular Th2-type inflammation and bronchoconstriction-induced remodelling.

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